Biol Blood Marrow Transplant 19 (2013) 298–304

Thrombotic Microangiopathy Associated with Sirolimus Level after Allogeneic Hematopoietic Cell Transplantation with Tacrolimus/Sirolimus-Based Graft-versus-Host Disease Prophylaxis

Sepideh Shayani [1,†], Joycelynne Palmer [2,†], Tracey Stiller [2], Xueli Liu [2], Sandra H. Thomas [3], Tam Khuu [1], Pablo M. Parker [3], Samer K. Khaled [3], Stephen J. Forman [3], Ryotaro Nakamura [3,*]

[1] *Pharmacy Department, City of Hope, Duarte, California*
[2] *Division of Biostatistics, City of Hope, Duarte, California*
[3] *Division of Hematology/HCT, City of Hope, Duarte, California*

Article history:
Received 26 April 2012
Accepted 10 October 2012

Key Words:
Calcineurin inhibitors
Schistocytes
Thrombocytopenia

A B S T R A C T

Posttransplantation thrombotic microangiopathy (TMA) is a multifactorial complication of allogeneic hematopoietic cell transplantation (allo-HCT) whose incidence is increased with the use of a sirolimus plus tacrolimus (SIR/TAC) regimen for acute graft-versus-host disease (aGVHD) prophylaxis. We evaluated the incidence and possible risk factors for TMA in a case series of 177 patients who received allo-HCT using SIR/TAC-based GVHD prophylaxis. The patients received either a sibling donor graft (n = 82) or a matched unrelated donor graft (n = 95). Within the first 100 days post-HCT, 30 patients (17%) were diagnosed with TMA, and an additional 9 patients (5%) were classified as probable TMA cases. The median time to onset of TMA was 4.6 weeks (range, 1.6-10.6 weeks). Thirty-four patients developed both TMA and aGVHD, with the majority (81%) developing aGVHD first. Multivariate analysis identified the following factors as associated with increased risk of TMA: day 14 serum sirolimus level ≥9.9 ng/mL (hazard ratio [HR], 2.19; 95% confidence interval [CI], 1.13-4.27; $P = .02$), presence of previous aGVHD grade II-IV (HR, 3.04; 95% CI, 1.38-6.71; $P < .01$), and fully myeloablative conditioning (HR, 3.47; 95% CI, 1.60-7.53; $P < .01$). These risk factors for TMA suggest that when using a SIR/TAC regimen for GVHD prophylaxis, careful monitoring and adjustment of the sirolimus dosage is critical, particularly in patients with active aGVHD.

INTRODUCTION

Posttransplantation thrombotic microangiopathy (TMA) is a serious complication of hematopoietic stem cell transplantation (HCT), characterized by the presence of arteriolar thrombi associated with damaged vessel walls. These thrombi lead to intravascular platelet activation and formation of platelet-rich thrombi within the microcirculation, causing platelet consumption, mechanical damage to blood cells, and organ damage [1]. Post-HCT TMA has been attributed to the vascular endothelial damage caused by radiation therapy, high-dose chemotherapy, immunosuppressive agents, graft-versus-host disease (GVHD), or infections [2]. Unlike most cases of familial or acquired thrombotic thrombocytopenic purpura, bone marrow transplantation–associated TMA is not generally associated with severely reduced or absent plasma metalloprotease ADAMTS13 activity [3]. With a few exceptions, plasma exchange is ineffective in treating TMA [3].

Diagnosing TMA in HCT recipients has proven challenging, owing to the presence of multiple contributing factors including opportunistic infections, delayed engraftment, medications, regimen-related toxicity, presence of acute GVHD (aGVHD), and other disorders associated with the HCT process. In 2005, the Toxicity Committee of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) proposed an operational definition of TMA (Table 1). Subsequently, an International Working Group was formed to develop a consensus set of criteria for diagnosing TMA [4] (Table 1).

Calcineurin inhibitors, such as cyclosporine and tacrolimus, may contribute to the development of TMA by increasing the production of thromboxane A_2 and decreasing production of prostacyclin, as well as by directly damaging renal endothelial cells. These agents also can cause neurotoxicity, another feature of TMA [1,2,5]. Recent studies have demonstrated that adding sirolimus to calcineurin inhibitors increases the risk of TMA after HCT [6,7]. The etiology of sirolimus-induced TMA is not clear, but may be related to enhanced platelet activation and aggregation, leading to endothelial damage. Another theory involves the pharmacokinetic interaction between sirolimus and calcineurin inhibitors, which might lead to increased serum and kidney levels of these agents.

In the present retrospective analysis, we sought to extend our earlier observations [7] and determine the incidence and clinical characteristics of post-HCT TMA associated with sirolimus and tacrolimus (SIR/TAC)-based GVHD prophylaxis. In addition, we evaluated the association between TMA and SIR/TAC serum levels in detail.

PATIENTS AND METHODS

Study Design

Between January 2005 and August 2007, a consecutive case series of 177 patients underwent allogeneic HCT (allo-HCT) at City of Hope using

Financial disclosure: See Acknowledgments on page 303.

* Correspondence and reprint requests: Ryotaro Nakamura, MD, City of Hope, 1500 E Duarte Road, Duarte, CA 91010.
E-mail address: rnakamura@coh.org (R. Nakamura).
† Sepideh Shayani and Joycelynne Palmer contributed equally to this work.

1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2012.10.006

Table 1
Diagnostic Criteria for TMA

Criterion	City of Hope*	CIBMTR	BMT CTN	International Working Group
Hemolysis	Presence of schistocytes, persistent nucleated RBCs	Microangiopathic hemolysis	RBC fragmentation and ≥2 schistocytes/high-power field on peripheral smear; negative direct and indirect Coombs test	Presence of (>4%) schistocytes in the blood; decreased haptoglobin or increased RBC transfusion requirement
Thrombocytopenia	Prolonged or progressive thrombocytopenia (platelets $<50 \times 10^9$/L or ≥50% decrease from previous counts)	Thrombocytopenia (platelets $<50 \times 10^9$/L)		Prolonged or progressive thrombocytopenia (platelets $<50 \times 10^9$/L or ≥50% decrease from previous counts)
Liver function	LDH >2 times upper limit of normal	LDH >2 times upper limit normal; bilirubin <2 times upper limit of normal	Concurrent increase in LDH above institutional baseline	Sudden, persistent increase in LDH
Renal function	SCr >1.5 times baseline	SCr >2 mg/dL or >50% over baseline	Concurrent renal and/or neurologic dysfunction with no other explanation	
Neurologic		Neurologic changes	Concurrent renal and/or neurologic dysfunction with no other explanation	

CIBMTR indicates Center for International Blood and Marrow Transplant Research; LDH, lactate dehydrogenase.
* The presence of all 4 City of Hope criteria listed defines definite TMA. Probable TMA is defined as concomitant presence of 3 of the 4 criteria in the presence of clinical diagnosis by an independent attending physician.

a SIR/TAC-based GVHD prophylactic regimen and hematopoietic stem cells from either a matched related sibling or a matched unrelated donor (MUD). Patients were identified and selected for retrospective analysis from a prospective observational research database. The City of Hope Institutional Review Board approved the analysis of these data. The patients with a matched related donor were previously reported as part of a phase I/II study [7].

Conditioning and GVHD Prophylaxis Regimens

Conditioning regimens included fludarabine (Flu)/melphalan (Mel)in 106 patients (59.9%), fractionated total body irradiation (FTBI)/cyclophosphamide (Cy) in 11 patients (6.2%), FTBI/etoposide in 46 patients (26.0%), and busulfan (Bu)/Cy in 14 patients (7.9%). Of these regimens, only Flu/Mel was classified as a reduced-intensity regimen. Patients were selected for a reduced-intensity regimen based on advanced age or the presence of other comorbidities in accordance with institutional protocol. GVHD prophylaxis was administered as described previously [7,8], as follows: sirolimus 12 mg orally on day −3 (loading dose), followed by 4 mg/day orally, with subsequent dose adjustments to maintain serum level at 3-12 ng/mL. Tacrolimus was initially dosed at 0.02 mg/kg/day i.v. starting on day −3, then switched to an equivalent oral dose when oral intake was adequate, to maintain a target serum level of 5-10 ng/mL. Serum sirolimus and tacrolimus levels were measured at least weekly until day +100, with dosage adjustments made for target levels and/or clinical toxicity. For MUD HCT with a <10/10 HLA-matched donor, methotrexate 5 mg/m^2 was added on days +1, +3, and +6. One patient undergoing MUD-HCT also received antithymocyte globulin 0.5 mg/kg on day −3, 1.5 mg/kg on day −2, and 2.5 mg/kg on day −1.

Supportive Care

Supportive care, including prophylactic antibiotics, antifungals, total parenteral nutrition, hematopoietic growth factors, immune globulin replacement, and treatment of mucositis and neutropenic fever, was administered in accordance with institutional protocols. All patients received acyclovir 250 mg/m^2 starting on day −1. Antifungal prophylaxis consisted of low-dose lipid complex amphotericin B (Abelcet; 1 mg/kg), caspofungin, or micafungin starting on day 0 or day +1. In addition, an oral azole antifungal (eg, posaconazole, voriconazole, fluconazole, itraconazole) was later added in selected patients for prophylaxis or treatment of fungal infections. Sinusoidal obstructive syndrome (SOS) prophylaxis was provided with low-dose heparin (100 U/kg/day) or ursodiol 300 mg twice daily.

Definition of TMA

We evaluated patients for TMA based on the criteria used at City of Hope. Our criteria are compared with other commonly used criteria in Table 1. Patients were diagnosed with definite TMA if they met the following diagnostic criteria: increased serum creatinine (sCr) level ≥50% above baseline, lactate dehydrogenase >2 times the upper limit of normal, the presence of schistocytes or persistent presence of nucleated RBCs, and prolonged or progressive thrombocytopenia (<50 x 10^9/L or ≥ 50% decrease). Patients were classified as having probable TMA if they experienced 3 of the 4 aforementioned criteria. Patients with characteristics of TMA secondary to disease relapse or progression were not classified as TMA cases in this study.

Definitions of GVHD and Response to Intervention

aGVHD was defined and staged according to Glucksberg et al. [9]. Chronic GVHD (cGVHD) was defined and staged by the limited/extensive classification scheme [10]. The response of TMA to intervention was defined as follows: complete response (CR), resolution of all TMA criteria to patient baseline; partial response (PR), improvement in individual TMA criteria such that the patient no longer meets the definition of TMA but has not returned to baseline levels.

Statistical Analysis

Demographic, disease, and treatment characteristics were summarized using descriptive statistics. Survival estimates were calculated based on the Kaplan-Meier product-limit method [11]; 95% confidence intervals (CIs) were calculated using the logit transformation and the Greenwood variance estimate [12]. Differences between Kaplan-Meier curves were assessed using the log-rank test. Patients who were alive at the time of analysis were censored at the last contact date. Overall survival (OS) was measured from transplantation to death from any cause. Event-free survival (EFS) was defined as the time from transplantation to disease recurrence, disease progression, or death. The relapse/progression incidence was defined as time from transplantation to disease recurrence or progression. The cumulative incidence of relapse/progression was computed treating a non-relapse death event as a competing risk, as described by Gooley et al. [13]. Nonrelapse mortality (NRM) was measured from the time of transplantation to death from any cause other than disease relapse or progression. The cumulative incidence of NRM was calculated using relapse/progression incidence as a competing risk. The cumulative incidence of aGVHD and hazard ratio (HR) were estimated after taking into account the competing risk of engraftment failure and early death/relapse. Time to onset of cGVHD was estimated accounting for the competing risks of early death or second transplant. Similarly the cumulative incidence of TMA was estimated after accounting for the competing risk of death and relapse. Possible differences between cumulative incidence curves in the presence of a competing risk were tested using the Gray method [14]. The significance of disease and treatment features on TMA risk and on NRM risk was assessed using the Cox proportional hazards regression analysis competing risks analogue [15].

In addition, a joint model [16] of tacrolimus/sirolimus serum levels and time to TMA was examined to consider the shared evolution of the repeated drug level measurements and event times, when a possible association between the two processes exists. Square-root transformed sirolimus and tacrolimus serum levels were used to reduce the variability of individual measurements and improve normality of the data. A desirable feature of the joint modeling approach is that when an association is absent, the joint analysis will produce the same results as would be obtained by performing separate analyses for each process.

An exploratory “drug level” analysis was also performed using the calculated median values for both tacrolimus and sirolimus levels over set durations (7, 14, and 30 days) post-HCT for each patient. For example, the

day 14 sirolimus level was the median value for the patient over the 14 days post-HCT (twice weekly levels, so 4 levels). Based on our published SIR/TAC experience, as part of a phase II trial using sibling donors, we hypothesized that SIR/TAC serum levels in the early period post-alloHCT (first 30 days) would be the most predictive of TMA outcome, even for those patients who maintain serum levels considered therapeutic/nontoxic. Thus, we conducted serum level evaluations for modeling at days 7, 14, and 30 days, among the upper quartiles of each of the distributions (equal to or greater than median values) for both tacrolimus and sirolimus.

TMA endpoints (eg, definitive, definitive/probable) were modeled as a function of prognostic variables that were determined by a literature review identifying factors associated with development of TMA in patients treated with allo-HCT. Factors evaluated for association with outcome included patient age at transplantation (<46 or ≥46 years), patient/donor sex match (female donor to male patient; others), disease risk status at transplantation (low, intermediate, or high; defined in Table 2), patient/donor cytomegalovirus status, conditioning regimen (Flu/Mel, FTBI/etoposide or Cy, or Bu/Cy; reduced intensity or myeloablative), donor type (related or unrelated), and aGVHD grade (0-I or II-IV). aGVHD grade II-IV was treated as a time-dependent covariate in the risk factor analysis for TMA. All calculations were performed using SAS version 9.2 (SAS Institute, Cary, NC) and R version 2.11.1 (http://www.r-project.org). Statistical significance was set at the $P < .05$ level; all P values were 2-sided. The data were locked for analysis on July 31, 2010 (analytic date).

RESULTS

This study comprises a consecutive case series of 177 patients who underwent allo-HCT at City of Hope between January 2005 and August 2007. Eighty-two patients (46%) received stem cells from a sibling donor, and 95 patients underwent matched unrelated HCT. The median patient age was 46 years (range, 10-70 years). Patient, disease, and transplantation characteristics are summarized in Table 2.

Overall HCT Outcomes

At a median follow-up of 50 months, 89 patients (49.5%) were alive. Of the 88 patient deaths, 42 were due to disease relapse/progression, and the remaining 46 were due to non–relapse-related causes. The median time to neutrophil engraftment was 16 days; 4 patients failed to engraft. The OS, EFS, and cumulative incidence of relapse/progression at 2 years were 59.9% (95% CI, 55.4%-64.1%), 51.7% (95% CI, 47.8%-55.5%), and 31.1% (95% CI, 25.0%-38.7%), respectively. NRM was 7.4% (95% CI, 4.4%-12.4%) at 100 days and 15.8% (95% CI, 11.3%-22.2%) at 2 years. The cumulative incidence of aGVHD grade II-IV was 50.5% (95% CI, 43.2%-59.1%), with a median onset of 23 days (Figure 1A). The cumulative incidence of grade III-IV aGVHD was 17.9% (95% CI, 10.6%-30.2%). The cumulative incidence of cGVHD (ie, time to cGVHD with competing risks of early death or second transplantation) was 62.5% (95% CI, 55.6-70.3%) at 1 year and 71.3% (95% CI, 64.6-78.7%) at 2 years.

Incidence and Timing of TMA

TMA data are summarized in Table 3. Of the 177 patients studied, 30 (17%) were diagnosed with definite TMA based on the institutional diagnostic criteria. In addition, 9 patients did not meet the definitive diagnostic criteria owing to a missing test, but were clinically diagnosed with TMA by an independent attending physician (ie, probable TMA). If those patients with probable TMA were included, the overall incidence of TMA in this study would be 22% (39 of 177) (Figure 1B). Seven of these 39 patients (17.5%) met the criteria for definitive/probable TMA in the setting of ongoing multiorgan failure. The median time from HCT to onset of definite TMA was 4.6 weeks (range, 1.6-10.6 weeks). TMA outcomes are presented in Table 3.

Table 2
Patient, Disease, and Transplantation Characteristics (n = 177)

Variable	Value
Patient sex, n (%)	
Female	79 (44.6)
Male	98 (55.4)
Patient/donor sex match, n (%)	
Male/female	40 (22.6)
Others	137 (77.4)
Age at transplantation, years, median (range)	46 (10-70)
Disease risk status at transplantation, n (%)*	
Low risk	69 (39.0)
Intermediate risk	33 (18.6)
High risk	75 (42.4)
Diagnosis, n (%)	
Acute myelogenous leukemia	63 (35.6)
Acute lymphoblastic leukemia	39 (22.0)
Non-Hodgkin lymphoma	25 (14.1)
Myelodysplastic syndrome	17 (9.6)
Chronic myelogenous leukemia	9 (5.1)
Myeloproliferative disorder	9 (5.1)
Hodgkin's lymphoma	8 (4.5)
Multiple myeloma	4 (2.2)
Chronic lymphocytic leukemia	3 (1.7)
Donor, n (%)	
Sibling	82 (46.3)
Unrelated	95 (53.7)
Stem cell source, n (%)	
Bone marrow	23 (13.0)
Peripheral blood	154 (87.0)
Cytomegalovirus serostatus, n (%)	
Patient-positive/donor-positive	75 (42.4)
Patient-positive/donor-negative	51 (28.8)
Patient-negative/donor-positive	25 (14.2)
Patient-negative/donor-negative	26 (14.7)
Conditioning regimen, n (%)†	
Flu/Mel	106 (59.9)
FTBI/Cy	11 (6.2)
FTBI/etoposide	46 (26.0)
Bu/Cy	14 (7.9)

* Disease risk status categories: low risk, all diseases in first complete remission, myeloma in partial remission, chronic myelogenous leukemia (CML) in first chronic phase, refractory anemia, and refractory anemia with ringed sideroblasts; intermediate risk, lymphoma/leukemia in second complete remission or partial remission, CML in second chronic phase or accelerated phase, myeloproliferative disorders; high risk, all diseases in relapse, induction failure or progressive disease, CML blast crisis, refractory anemia with excess blasts.

† Of the conditioning regimens, only Flu/Mel was reduced intensity; all others were fully myeloablative.

Management of TMA and Outcomes

TMA interventions and responses are detailed in Table 4. Initial treatment for patients diagnosed with definite TMA (n = 30) included discontinuation of sirolimus (n = 9), tacrolimus (n = 8), or both (n = 6) and immunosuppressant dosage adjustments for the remaining 7 patients. At the time of resolution, tacrolimus and sirolimus were both discontinued in 12 patients, and only 4 patients remained on the combination. Sirolimus alone was continued for 11 patients, whereas tacrolimus alone was continued in only 3 patients. Of the 30 patients diagnosed with TMA, 8 were treated with plasma exchange at the discretion of the treating physician, and 4 of these patients responded. Overall, 22 patients (73%) responded to treatment, with a complete resolution rate of 70%. The median time from diagnosis of TMA to response and resolution of symptoms was 4.5 and 5.5 weeks, respectively (range, 1-27 weeks). Twenty-five patients had both tacrolimus and sirolimus discontinued and were managed with mycophenolate mofetil (MMF) plus prednisone (n = 15), prednisone alone (n = 9), or MMF alone (n = 1). One patient was discontinued from sirolimus but remained on tacrolimus alone.

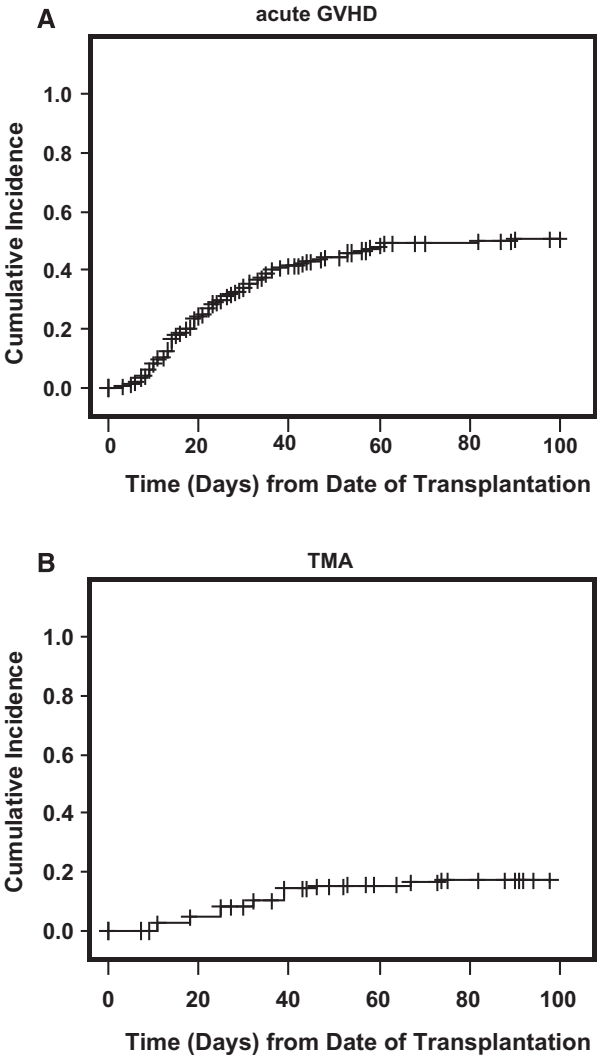

Figure 1. Cumulative incidence of aGVHD and TMA in the first 100 days posttransplantation. (A) Time to onset of grade II-IV aGVHD. (B) Time to onset of definitive TMA, with death before 100 days calculated as a competing risk.

Survival outcomes were evaluated in the patients with TMA and those without TMA. The cumulative incidence of NRM at 2 years was 33.3% in those with TMA, compared with 12.25% in those without TMA ($P = .004$) (Figure 2). According to the multivariate model for NRM, after adjusting for conditioning regimen, aGVHD, and serum sirolimus level, TMA was independently associated with increased NRM (HR, 2.76; 95% CI, 1.29-5.92; $P = .009$). Multivariate results for NRM are summarized in Table 5.

Table 3
TMA Outcomes

Variable (n = 30 for Definite TMA)	Value
TMA incidence, %	17 (30 of 177 patients)
Time to TMA onset, weeks, median (range)	4.6 (1.6-10.6)
TMA with aGVHD (any time), %	90 (27 of 30 TMA cases)
TMA with previous aGVHD, %	81 (22 of 27 cases with aGVHD)
TMA response to treatment	
Response, %	73 (22 of 30 TMA cases)
Time to response, weeks, median	4.5 (1-27)
Complete resolution, %	70 (21 of 30 TMA cases)
Time to complete resolution, weeks, median (range)	5.5 (1-27)
Maximum LDH	1128-62,817
Median LDH	2230
Maximum creatinine	0.6-4.5
Median creatinine	1.5

Table 4
Response of TMA to SIR/TAC Intervention

Intervention	Initial Intervention	Final Intervention
Discontinued sirolimus, responded/treated	9/9	11/11
Discontinued tacrolimus, responded/treated*	5/8	3/3
Discontinued tacrolimus and sirolimus, responded/treated†	2/6	5/12
Dosage modification, responded/treated*	6/7	3/4

Values are the number of patients responding to a treatment over the number treated. The initial intervention shows the first treatment responses in each category; final intervention, results of the last treatment attempted.
* One patient was treated with dosage reduction of sirolimus and discontinuation of tacrolimus and plasma exchange, and responded to this therapy.
† Seven patients were treated with discontinuation of both tacrolimus and sirolimus as well as plasma exchange, and 3 patients responded to this overall therapy.

Acute GVHD and Use of Azoles in Patients with TMA

The overall cumulative incidence of aGVHD (grade II-IV) was 50.5% (95% CI, 43.2%-59.1%). Among the 30 patients who developed definite TMA, 27 (90%) also developed aGVHD, with the majority (81%) developing TMA after aGVHD had been diagnosed. Of the 30 patients with definitive TMA, 14 did not receive an azole, 15 received an azole (3 posaconazole in 3, itraconazole in 5, and voriconazole in 7) before TMA was diagnosed, and 1 received voriconazole at the time of TMA diagnosis.

High Sirolimus Level Associated with TMA

Factors identified on univariate Cox regression analysis as significantly associated with TMA at the $P = .10$ level—conditioning regimen, presence of aGVHD, serum sirolimus level at day +14—were included in the

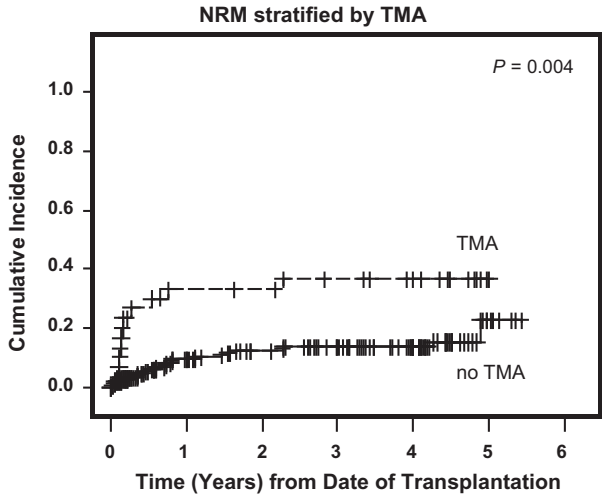

Figure 2. NRM stratified by presence of TMA. Time to death from nonrelapse causes calculated with relapse, progression, or second transplantation as competing risks. The dashed line represents patients with definitive TMA (based on laboratory criteria).

Table 5
HR for Risk of NRM and TMA

Parameter	Value	No. of Patients	No. of Events	HR (95% CI)	*P* Value
NRM risk					
Sirolimus, 14-day level, ng/mL	<9.9	132	21	Baseline	
	≥9.9	44	12	1.67 (0.79-3.51)	.18
TMA	No TMA	146	22	Baseline	
	Definitive TMA	30	11	2.76 (1.29-5.92)	.009
TMA risk					
Conditioning	Reduced intensity	106	9	Baseline	
	Fully myeloablative	70	21	3.47 (1.60-7.53)	.002
aGVHD	Grade 0-I	95	8	Baseline	
	Grade II-IV	81	22	3.04 (1.38-6.71)	.006
Sirolimus, 14-day level, ng/mL	<9.9	131	18	Baseline	
	≥9.9	45	12	2.19 (1.13-4.27)	.02

Reduced-intensity conditioning included only the Flu/Mel regimen; all other regimens were fully myeloablative.

multivariate model. Results of the multivariate analysis are displayed in Table 5. The highest quartile of serum sirolimus exposure on day 14 (cutoff, 9.9 ng/mL) was found to be independently associated with an increased risk of definitive TMA (HR, 2.19, 95% CI, 1.13-4.27; $P = .02$). Other significant risk factors were previous aGVHD grade II-IV and use of a fully myeloablative conditioning regimen (Table 5). Tacrolimus levels were not significantly associated with TMA. Median drug levels over the first 30 days after allo-HCT were not found to be predictive of TMA overall.

Because sirolimus binds to the same family of intracellular FK-506 (tacrolimus)-binding proteins at a site distinct from tacrolimus, these 2 drugs may interact or, when used in combination, produce a total effect greater than the sum of their individual effects. We further evaluated the possible impact of tacrolimus and sirolimus levels on TMA risk by constructing joint models of the repeated drug level measurements over time and time-to-event (TMA) data. The joint models included both additive (model 1) and multiplicative (model 2) parameters for sirolimus and tacrolimus (SIR + TAC, SIR × TAC), with and without other covariates (eg, age, type of conditioning regimen). The additive joint model showed a marginally significant drug-level effect (association, $P = .06$ for definite TMA; Supplemental Table 1), suggesting that TMA risk also might be affected by the additive effects of the 2 drugs beyond the individual effects of sirolimus, as demonstrated on multivariate analysis. This marginal significance remained after adjusting for other covariates, age, and conditioning regimen ($P = .07$ for definite TMA; Supplemental Table 2). The results of the joint multiplicative model were not significant.

DISCUSSION

Our data extend earlier observations, including those from City of Hope and Dana-Farber Cancer Institute, that SIR/TAC-based GVHD prophylaxis is associated with an increased risk of TMA. With increased numbers of patients and more detailed drug level analysis, our data corroborate that serum sirolimus level is one of the risk factors associated with TMA.

Diagnosis of TMA is difficult in patients who have undergone HCT, who commonly develop thrombocytopenia, anemia, renal dysfunction, and RBC fragmentation post-transplantation [17]. Schistocytes occur in a variety of conditions, including chronic renal failure, preeclampsia, and prosthetic heart valve implantation. The presence of up to 4% schistocytes in the absence of other TMA hallmarks also has been reported in patients receiving marrow allografts or autografts for various indications, which puts these patients at risk for misdiagnosis of TMA [3]. Cho et al. [18] validated criteria for TMA proposed by the BMT CTN [17] and the International Working Group (IWG) [4], in an analysis of 672 allogeneic HCT recipients. In their study, the incidence of TMA by BMT CTN–defined criteria was 6.1%, compared with an incidence of only 2.5% by the IWG definition. These authors reported an overall cumulative incidence of TMA of 12.7%, which included both definite TMA cases (by BMT CTN criteria) and probable TMA cases (meeting BMT CTN criteria without renal or neurologic dysfunction). Two-thirds of the patients defined as having TMA by the BMT CTN criteria had no schistocytes, which is required by the IWG criteria, and 18% of the patients classified with TMA based on the IWG criteria did not have renal or neurologic dysfunction.

In the present study, we used institutional criteria for TMA (Table 1). We did not set a quantitative criterion for schistocytes, because precise quantification is difficult, and the presence of any schistocytes in the setting of hemolysis is considered sufficient to indicate suspected TMA. To capture a broad range of TMA or TMA-like pathology, we included nucleated RBCs as part of our TMA assessment, given our impression that nucleated RBCs tend to appear earlier than schistocytes and are more readily detected on routine CBC analyses. The vast majority (93%) of our patients with TMA presented with both nucleated RBCs and schistocytes. In our analysis, we also considered probable cases in which there was one missing test/criterion and/or the treating physician made a clinical diagnosis of TMA and intervened accordingly. Although we report results only for the definite TMA cases, we conducted all of the analyses including the probable TMA cases as well, and found qualitatively similar results. We included the probable cases because we believe that TMA is often underestimated in the literature. For example, at our institution, many probable cases were identified as TMA by the treating physician before the patient met all 4 criteria, to allow for earlier institution of treatment in a “preemptive strike” approach.

Recent studies, including our own study, have demonstrated a particularly high incidence of TMA after allo-HCT using sirolimus-containing GVHD prophylaxis [6-8]. Our present findings confirm and extend the previous results and more closely associate TMA with higher serum sirolimus levels, even levels believed to be therapeutic and nontoxic. Importantly, significant associations with sirolimus serum levels >9.9 ng/mL were observed for the early time points (posttransplantation day 14). Based on these data, we have modified our upper range of therapeutic sirolimus levels from the original 12 ng/mL to 10 ng/mL. Although our present analysis identified no direct impact of tacrolimus on TMA, computing tacrolimus levels together with sirolimus

levels using a square-root additive joint model revealed a trend toward an association with TMA ($P = .07$ by multivariate analysis). In other words, tacrolimus when given in combination with sirolimus may increase the risk of TMA beyond that associated with sirolimus alone. This issue merits additional study with a larger number of patients.

Serum levels of both tacrolimus and sirolimus are also influenced by genes affecting drug absorption, distribution, metabolism, and excretion (ADME). Allelic variants in ADME genes can determine the pharmacokinetic variability of medications and have been shown to influence the outcomes of medical treatments using these drugs for kidney transplantation. We are currently investigating the effects of ADME variants in HCT recipients with the goal of better tailoring the initial doses of these drugs to avoid toxicities, including TMA.

We also identified other TMA risk factors, including fully myeloablative conditioning regimens and previous occurrence of aGVHD grade II-IV. Concurrent GVHD has been reported as a risk factor by the Dana-Farber Cancer Institute [6] and others [19]. Bu/Cy conditioning has been previously associated with TMA and SOS, but we found no independent association of Bu/Cy with incidence of TMA. Rather, fully myeloablative conditioning was independently significantly associated with TMA, even when patients receiving Bu/Cy were excluded from the analysis. The addition of methotrexate to sirolimus has been shown to increase the risk of SOS [20]. In the present study, all recipients of MUD grafts received methotrexate, yet there was no significant difference in the incidence of TMA between recipients of sibling donor grafts and recipients of MUD donor grafts in this cohort of patients. Patients who developed TMA demonstrated an independently significant increased risk of NRM, highlighting the need to better understand and prevent this complication of HCT.

A potential risk factor that we would like to pursue in a prospective study is the role of previous azole antifungal agents. Given this study's retrospective nature, detailed data on azole use were not collected for all patients, but they were obtained for the TMA cases. Half of the patients with definitive TMA had previous exposure, which is high for our practice, in which primary prophylaxis includes nonazole antifungal medications. Because azoles are commonly used for patients with GVHD requiring high-dose steroids, an independent effect of azoles is difficult to analyze; thus, we opted to exclude this factor in our Cox model.

There are no standardized treatments for post-HCT TMA. Given that plasma exchange has not been shown to provide a significant benefit in treating patients with TMA and is associated with major complications [21], most patients were not treated with this modality. Discontinuation of tacrolimus and/or sirolimus was the primary means of treatment for TMA and three-quarters of the patients improved in this study. However, survival outcomes were significantly different between those who developed TMA and those who did not (Figure 2). In the majority of patients who died without relapse after experiencing TMA, the cause of death was associated with GVHD/infection, many of which met the definition of TMA in the context of multiorgan failure. Thus, based on the present study, it cannot be concluded that the occurrence of TMA is an independent predictor of poor survival.

Our data should be interpreted with caution. The observed associations between sirolimus levels and TMA may be related to hidden confounding factors in this heterogeneous cohort. Although sirolimus levels were checked at trough levels and in a consistent manner in our program, there is inherent variability in the exact timing of the blood draws and patients' compliance with instructions in an outpatient setting. To minimize this variability, we focused our multivariate analysis on the early post-HCT period (within 30 days), during which most drug level measurements were obtained in the inpatient setting. Our data indicate a dose–response relationship between the use of sirolimus and the occurrence of TMA, and suggest that SIR/TAC therapy may exacerbate this effect. The currently accepted sirolimus therapeutic ranges may be too high, contributing to unacceptable toxicity in some patients, particularly those with concurrent GVHD or a genetic predisposition based on ADME genes.

Our institutional guidelines have been modified to maintain both sirolimus and tacrolimus levels in the 5-10 ng/mL range whenever they are used in combination. We previously allowed 3-12 ng/mL for sirolimus, but have amended this range based on data from the present study and work by Rodriguez et al. [7]. In addition, in the event of fungal infection, if azoles are used, we recommend dosage reduction of tacrolimus and sirolimus to prevent supratherapeutic levels secondary to drug–drug interaction. For patients with active GVHD who develop TMA, based on the severity of TMA, we recommend dosage adjustment or discontinuation of sirolimus or of both sirolimus and tacrolimus. Other immunosuppressive agents, such as steroids and mycophenolate mofetil, are typically used in the event of discontinuation.

ACKNOWLEDGMENTS

We thank the dedicated medical and administrative staff of City of Hope, without whom this work would not have been possible. This study was supported by National Institutes of Health grants P30 CA33572 and P01 CA30206.

Financial disclosure: The authors have no conflicts of interest to disclose.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.10.006.

REFERENCES

1. Batts ED, Lazarus HM. Diagnosis and treatment of transplantation-associated thrombotic microangiopathy: real progress, or are we still waiting? *Bone Marrow Transplant*. 2007;40:709-719.
2. Sadler JE, Poncz M. Antibody-mediated thrombotic disorders: thrombotic thrombocytopenic purpura and heparin induced thrombocytopenia. In: Kaushansky K, Lichtman M, Beutler E, et al., editors. *Williams Hematology*, 8th ed. New York: McGraw-Hill; 2010. p. 2163-2183.
3. Sadler JE, Moake JL, Miyata T, et al. Recent advances in thrombotic thrombocytopenic purpura. 2004. *Hematology*. 2004;407-423.
4. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica*. 2007;92:95-100.
5. Chohan R, Vij R, Adkins D, et al. Long-term outcomes of allogeneic stem cell transplant recipients after calcineurin inhibitor-induced neurotoxicity. *Br J Haematol*. 2003;123:110-113.
6. Cutler C, Henry NL, Magee C, et al. Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11:551-557.
7. Rodriguez R, Nakamura R, Palmer JM, et al. A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. *Blood*. 2010;115:1098-1105.
8. Cutler C, Kim HT, Hochberg E, et al. Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophylaxis after matched

related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant*. 2004;10:328-336.
9. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation*. 1974;18:295-304.
10. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
11. Kaplan G, Meier P. Non-parametric estimations from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
12. Breslow NE, Day NE, editors. *Statistical Methods in Cancer Research: Volume II, The Design and Analysis of Cohort Studies. IARC Scientific Publication 82*. Lyon, France: IARC; 1987. p. 177-229.
13. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
14. Gray RJ. A class of *k*-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1140-1154.
15. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496-509.
16. Rizopoulos D. JM: An R package for the joint modeling of longitudinal and time-to-event data. *J Stat Software*. 2010;35:1-33.
17. Ho VT, Cutler C, Carter S, et al. Blood and Marrow Transplant Clinical Trials Network Toxicity Committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11:571-575.
18. Cho B-S, Yahng S-A, Lee S-E, et al. Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. *Transplantation*. 2010;90: 918-926.
19. Fuge R, Bird JM, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. *Br J Haematol*. 2001;113:58-64.
20. Cutler C, Stevenson K, Kim HT, et al. Sirolimus is associated with veno-occlusive disease of the liver after myeloablative allogeneic stem cell transplantation. *Blood*. 2008;112:4425-4431.
21. George JN, Li X, McMinn JR, et al. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. *Transfusion*. 2004;44: 294-304.